STUDIES OF GAS AND ELECTROLYTE EQUILIBRIA IN BLOOD.

I. TECHNIQUE FOR COLLECTION AND ANALYSIS OF BLOOD, AND FOR ITS SATURATION WITH GAS MIXTURES OF KNOWN COMPOSITION.


(From the Hospital of The Rockefeller Institute for Medical Research.)

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INTRODUCTION.

Recent articles by L. J. Henderson (1920, 1921), Parsons (1919), Van Slyke (1921, b; 1922), and A. V. Hill (1922) have summarized the present state of our knowledge concerning the interaction of oxygen, carbon dioxide, hemoglobin, chlorides, and other constituents involved in the respiratory function of the blood and in the maintenance of its neutrality and osmotic pressure. The field in its present state has been developed from the blood gas studies of Bohr, Haldane, Barcroft, and their collaborators, and the investigations of the blood electrolytes dating from Zuntz through Hamburger and Gürber to present investigators (e.g. L. J. Henderson, 1908; Van Slyke and Cullen, 1917; Adolph and Ferry, 1921; Fridericia, 1920; Doisy, Eaton, and Chouke, 1922; Barcroft, Bock, Hill, Parsons, Parsons, and Shoji, 1922).

The reactions known to be involved in the respiratory changes of the blood, and the accompanying shifts of gases and acids between plasma and cells, are in part indicated qualitatively by the accompanying diagram (Fig. 1).

All six reactions are forced from left to right by increase in H$_2$CO$_3$, which results in formation in the plasma of bicarbonate from two sources (Reactions 1 and 2), and in the cells from two other sources (Reactions 5 and 6).
<table>
<thead>
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<tbody>
<tr>
<td>(1) ( \text{H}_2\text{CO}_3 + \text{NaProtein} \rightleftharpoons \text{HProtein} + \text{NaHCO}_3 )</td>
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<tr>
<td>(2) ( \text{H}_2\text{CO}_3 + \text{NaCl} \rightleftharpoons \text{NaHCO}_3 + \text{HCl} ) ( \rightarrow ) ( \text{HCl} \leftrightarrow )</td>
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<tr>
<td>( \text{H}_2\text{CO}_3 \leftrightarrow \text{H}_4\text{CO}_4 )</td>
<td></td>
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<td>( \text{O}_2 )</td>
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\[ (3) \quad \text{HCl} + \text{K}_2\text{HPO}_4 \rightleftharpoons \text{KH}_2\text{PO}_4 + \text{KCl} \]

\[ (4) \quad 2 \text{HCl} + 2\text{KHbO}_2 \rightleftharpoons 2 \text{KCl} + \begin{cases} \text{HHbO}_4 \\ \text{HHb} + \text{O}_2 \end{cases} \]

\[ (5) \quad \text{H}_2\text{CO}_3 + \text{K}_2\text{HPO}_4 \rightleftharpoons \text{KHCO}_3 + \text{KH}_2\text{PO}_4 \]

\[ (6) \quad 2 \text{H}_2\text{CO}_3 + 2 \text{KHbO}_2 \rightleftharpoons 2 \text{KHCO}_3 + \begin{cases} \text{HHbO}_4 \\ \text{HHb} + \text{O}_2 \end{cases} \]

**Fig. 1.**
The displacement of base from combination with hemoglobin by HCl (Reaction 4) or H$_2$CO$_3$ (Reaction 6) results in a decrease in the oxygen bound by hemoglobin (last steps of Reactions 4 and 6), because when alkali hemoglobinate loses part of its alkali it also loses part of its affinity for oxygen.

On the other hand, increase of $O_2$ (from the lungs) forces Reactions 4 and 6 backwards, and thereby through Reactions 6 and 2 sets H$_2$CO$_3$ free.

By the reactions outlined influx of oxygen sets H$_2$CO$_3$ free and thereby helps the blood throw off in the lungs its overload of CO$_2$; while in the capillaries influx of CO$_2$ sets oxygen free from combination and thereby makes it more readily available to the tissues.

It is apparent from the diagram that every reaction affects every other reaction. In a given blood the $O_2$ and the H$_2$CO$_3$ concentrations are the two variables that are directly changed by respiration, and their alterations govern the accompanying changes in all the other variables, which are dependent on them. As L. J. Henderson has pointed out (1921), when we have in a given blood only two independent variables to deal with, by determining the relationship of each other variable to these two, or to any given two within the system, the quantitative interrelationships among them all may be determined. This may be accomplished either algebraically, or, more simply, by a two dimensional diagram such as Henderson has used (1921), or by an alignment chart. (The pH, although not indicated on our diagram, is also one of these dependent variables, since its value is set by the BHCO$_3$ and H$_2$CO$_3$ according to Hasselbalch's (1917) equation $pH = pK' + \log \frac{[BHCO_3]}{[H_2CO_3]}$).

It appears that the chief substances and reactions involved in the respiratory changes of the blood are probably known; and that Henderson has solved the mathematical problem of expressing by a practicable method the many relationships involved.

One cannot, however, read the recent theoretical papers quoted at the beginning of this paper without being struck by the present inadequacy of experimental data sufficiently accurate and complete to permit within definable limits of error the formulation of the
quantitative relationships involved.\textsuperscript{1} Such data require simultaneous observations of several changes with technique of a precision gauged in detail to meet the requirements of the problem.

Preliminary experiments leading towards the systematic determination of data on the various factors in the system were begun by McLean, Murray, and L. J. Henderson (1920) in Henderson's laboratory. The magnitude of the task made a coordinated division of it between at least two laboratories advisable, and the present series of papers from this laboratory is the partial result of such a division.

The problem of the determination of CO\textsubscript{2} and O\textsubscript{2} tensions by analyses of the gas phase was already solved by Haldane's apparatus for air analysis. We used this apparatus with the single 4-way stop-cock introduced by Yandell Henderson (1918).

The methods available for the determination of blood gases were less satisfactory. Fig. 2 is constructed from observations collected from the literature by Peters, Barr, and Rule (1920), and presents in graphic form the relations which appear to obtain between the carbon dioxide tension, the carbon dioxide concentration, and the hydriion concentration in average normal human blood. On this figure have been introduced carbon dioxide absorption curves (a), of completely oxygenated blood; (b), of completely reduced blood (located at the distance above the curve of oxygenated blood indicated by the results of Christiansen, Douglas, and Haldane (1914)); and (c), of plasma from the oxygenated blood. A comparison of these curves shows the magnitude of the changes which it is necessary to analyze.

The large unblocked rectangle (A) represents an estimation of the maximum combined error involved in the determination of the carbon dioxide absorption curve by a technique employed recently by Peters, Barr, and Rule (1920). The error is indicated in terms of pH, of volumes per cent of carbon dioxide in the blood, and of millimeters of carbon dioxide tension. We believe that the procedure employed in these experiments was as accurate as that used by previous workers. Nevertheless, the rectangle representing the possible cumulative error covers 40 per cent of the difference

\textsuperscript{1} The quantitative relationships between Reactions 1 and 2 appear satisfactorily established in a paper by Doisy, Eaton, and Chouke (1922), which appears as this paper goes to press.
A = Maximum combined error estimated to exist in method used by Peters and Barr for the determination of the CO₂ absorption curve.

(2.5 vol. per cent)

B = Error ascribed by Hasselbalch to the electrometric method of determining pH.

(0.02)

C = Maximum error of earlier methods of measuring the carbon dioxide content of blood.

(1.0 vol. per cent)

D = Error of present electrometric pH measurements.

(0.01)

E = Small black square in center represents variations in duplicate determinations of CO₂ content by improved method now employed.

(±0.1 vol. per cent)
between the carbon dioxide content of completely reduced and that of completely oxygenated blood. The cross-hatched rectangle (C) represents the limits of variation in duplicate determinations of the blood carbon dioxide by the method presented by Van Slyke in 1917 which was used by Peters, Barr, and Rule. These limits are of the same magnitude as those usually ascribed to the Barcroft and Haldane methods. The difference between the clear rectangle (A) and the cross-hatched rectangle (C) indicates the sum of the errors introduced by the manipulations which preceded the analyses. Although these errors are only estimated, we believe the estimates are approximately correct.

By improvements in apparatus and procedure for blood gas determinations (Van Slyke and Stadie, 1921), it is now possible to obtain consistently duplicate carbon dioxide readings with an average variation from the mean of ± 0.1 volume per cent or ± 0.05 millimol, a value represented by the small solid black square. A more recent improvement² has reduced the maximum variation in results by a skilled analyst, to ± 0.05 millimol of either CO₂ or oxygen. This variation is of about the same magnitude as that entailed in the volumetric measurement of blood in a pipette calibrated to deliver 1 cc. of water.

Variations in chloride estimations may be reduced to ± 0.1 millimol by the application of the method of Austin and Van Slyke (1920, 1921) to sufficiently large samples of blood.

The analytical errors having thus been reduced, it remained to develop a procedure for preliminary treatment which could be reproduced so accurately that two specimens of blood subjected to it would not differ in composition from each other, or from their common curves, by more than the analytical errors. It is the purpose of this paper to present a technique for the preliminary treatment of blood which will meet these requirements.

Table I presents the steps in the procedure of an ordinary experiment, and the factors which are active in the production of error.

² The principle of this apparatus was published last year in a preliminary note (Van Slyke, 1921, a). The details will appear shortly by Van Slyke and Neill in this Journal.
### TABLE I.
Factors Involved in Determining Gas and Electrolyte Equilibria in Blood.

<table>
<thead>
<tr>
<th>Steps in procedure</th>
<th>Sources of error</th>
</tr>
</thead>
<tbody>
<tr>
<td>The drawing, preparation, and preservation of blood</td>
<td>Hemolysis.</td>
</tr>
<tr>
<td>The saturation of blood at a desired temperature with a desired gas mixture</td>
<td>Formation of non-volatile (lactic?) acid.</td>
</tr>
<tr>
<td>Determination of the exact composition of the gas mixture at equilibrium</td>
<td>Change of equilibrium conditions during separation of gas and liquid phases.</td>
</tr>
<tr>
<td>The delivery of blood from the tonometer into a receiving vessel</td>
<td>Change of gas content of blood by exposure to air or oil.</td>
</tr>
<tr>
<td>Separation of serum or plasma</td>
<td>Change of gas content by exposure.</td>
</tr>
<tr>
<td>Preservation of blood, plasma, or serum for analysis</td>
<td>Formation of non-volatile acid (whole blood only). Formation of CO₂ and consumption of O₂ (whole blood only).</td>
</tr>
<tr>
<td>Analyses of blood, plasma, or serum</td>
<td>Change of gas content by exposure during transfer of sample from container to apparatus for analysis. Uneven mixture of cells in whole blood at moment of measuring sample. Limit of accuracy of methods for analysis of gas and liquid phases.</td>
</tr>
</tbody>
</table>

### Sources of Error.

1. **Hemolysis.**—Hemolysis changes the distribution of gases and electrolytes between cells and plasma. We avoided hemolysis by careful handling, and by using for most of our animal experiments horse blood, the cells of which are less fragile than those of dog blood. In the case of dog blood, we generally used serum in place of plasma, when determinations on the cell-free fluid were required.
2. Formation of Non-Volatile Acid in Blood.—Christiansen, Douglas, and Haldane (1914) showed that if defibrinated human whole blood is kept at 37° its carbon dioxide capacity falls by as much as 2 volumes per cent in a half hour, apparently because of the formation of non-volatile acid. Peters, Barr, and Rule (1920) observed a similar acid formation in human blood, both defibrinated and oxalated, but found that when the blood was allowed to stand at room temperature after being drawn it showed no significant changes during the 1st hour.

The formation of acid is confined to the cells, since it does not occur in separated plasma. The time of onset varies in bloods of different species. Dog blood has been observed to change measurably in an hour at room temperature, while horse blood has shown no measurable change in several hours. In a paper which appears as this goes to press Evans (1922) suggests that the non-volatile acid is formed by glucolysis, since its rate of formation parallels that of glucose disappearance. He finds that the acid formation is accelerated by loss of CO₂ (increase in pH) and is retarded by the addition of 0.1 per cent of sodium fluoride.

Up to the present we have avoided error from acid formation by working with horse blood, and by chilling the blood to zero whenever any time was allowed to elapse between the withdrawal of the blood and its exposure to a gas mixture or between this exposure or centrifugalization and analysis. We have also used uniform periods for saturation at 38°, so that if acid formation should occur it would be relatively constant in different blood samples. As a control of this factor, in experiments which involved a long series of exposures of samples of a given blood, we have repeated the conditions of the first exposure on the last of the series. Even when the chilled horse blood was permitted to stand some hours between the first and last exposures, no change was noted.

3. Formation of CO₂ and Consumption of O₂ by Metabolism of Whole Blood.—It has been shown by Harrop (1919) that, by this process, oxalated normal human blood loses 0.1 to 0.4 volumes per cent of oxygen in 6 hours at 38°. In horse blood we have found no significant changes at room temperature in an hour, which was the maximum time that blood was allowed to stand unchilled between withdrawal or saturation and analysis.
4. Uniform Mixtures of Cells and Plasma.—The necessity of obtaining a uniform mixture before samples are taken for either analysis or saturation is especially great in horse blood, because the cells settle with unusual rapidity. The uniformity of mixture attained by our procedures was controlled by oxygen capacity determinations, and the procedures were regulated accordingly. The tubes in which blood was kept over mercury (Tube J, Fig. 3), for example, were inverted twenty times immediately before a sample was withdrawn.
Change of Equilibrium Condition During Separation of Gas and Liquid Phases.—Changes in temperature or pressure, such as may result when the tonometer is removed from the bath, may so disturb conditions of gaseous equilibrium existing in the tonometer as to render it impossible to obtain results sufficiently accurate for the purposes of the problem outlined above.

In the procedure we have usually used ("First saturation method" outlined below), the gas and liquid phases, in a state convenient for analysis, have been separated while the tonometer was still in the water bath by a mechanical device which avoided changes of temperature and pressure. In a second process, used for large amounts (over 30 cc.) of blood, the initial composition of the gas phase was fixed with especial care, and the amounts of CO₂ and O₂ taken from or given to it by the blood during saturation were calculated from accurate analyses of the blood before and after the saturation. Consequently analysis of the gas phases was avoided.

Collection and Preparation of Blood. (a). For Saturation without Previous Analysis.—When blood is to be exposed to known gas mixtures prior to all analyses, rigid precautions to avoid changes in gas content before exposure are, of course, not required. We have, however, avoided gross losses of carbon dioxide, such as might possibly cause irreversible changes, by drawing the blood under oil in cylinders arranged as described by Van Slyke and Cullen (1917, Fig. 1).

When oxalated blood was desired, potassium oxalate of tested neutrality was placed in the receiver in the proportion of 0.3 gm. to 100 cc. of blood. A saturated solution of neutral potassium oxalate was spread in a film on the walls of the receiver and dried by an air current. The neutrality of the oxalate was tested by the addition of phenol red to a diluted sample. Some samples of oxalate are alkaline. To such, oxalic acid was added until the pH was 7.4.

When defibrinated blood was required the oxalate was omitted, and the blood was defibrinated by stirring under oil with a rod. The blood was then filtered under oil through gauze.

(b). Collection and Centrifugation of Blood for Analysis.—When it was necessary to ascertain the gas content of the blood as drawn, as well as after exposure to a known gas mixture we either drew it
under oil by the above procedure, or used the apparatus shown in
Fig. 3. When small samples (10 to 20 cc.) were drawn under oil,
the precaution was taken to use rubber and glass tubes (the latter
Pyrex) of only 2 mm. diameter for the blood to pass through, so
that it would form a solid column without bubbles.

The receiving vessel, J, coated inside with oxalate, is connected with
the mercury bulb M and the 3-way cock H bearing the needle. J and its
connections up to H are filled with mercury, a few drops of which are wasted
through F. The needle is then inserted into the blood vessel, and a few
drops of blood are permitted to escape from F, in order to abolish the
air-space remaining in the connecting tube. By turning H (with M in the
lower position shown in Fig. 3) the needle and collecting vessel are directly
connected and the blood is drawn into the latter. We have used tubes of
the type J of from 10 to 200 cc. capacity.

As soon as the blood has been drawn J is inverted a number of times in
order to insure mixing of the blood and oxalate, a process which is assisted
by the mercury that remains. The leveling bulb M is kept at the upper
level, in order to keep positive pressure on the blood sample.

Sampling Blood.—The cells and plasma are thoroughly mixed
immediately before sampling by repeatedly inverting J. The
leveling bulb being in the upper ring, the pipette P is inserted into
the rubber tube outlet of J, the pinch-cock is opened, K is opened,
and blood is allowed to flow up into the pipette.

Centrifugation without Loss of CO₂.—We have used centrifuge
tubes of the type L in Fig. 3, similar to that described by Parsons
(1919). A Pyrex centrifuge tube is fitted with a 1-hole stopper
with a flange. Mineral oil is placed in the open tube, and the blood
is allowed to flow under the oil and displace all but the last drops of
oil from the tube. The stopper is then inserted, forcing out the
remaining oil through the hole in the rubber. The glass plug
is inserted in the hole, and the tube is ready for centrifugation.

After centrifugation the glass plug is removed, and a pipette
containing oil is inserted in the hole. When the stopper is
removed the oil flows over the surface of the plasma and prevents
the escape of CO₂ during transfer of the plasma to the sampling
tube J. For the protection needed, mineral oil is adequate,
since it is in contact with the blood only a short time during which
the blood is not agitated.

As an alternative to stoppering the centrifuge tube as above
described, the surface of the blood may be covered, after most of
the mineral oil has been removed, by a layer of melted paraffin (melting point 40-45°) which need not exceed 5 mm. in thickness. After centrifugation, a little mineral oil is poured over the paraffin. A transfer tube or pipette may then be inserted through or along side the paraffin to remove the plasma. This technique is useful if the amount of blood is too small to fill the centrifuge tube completely.

The effects of variations in treatment on the loss of CO₂ from solutions containing NaHCO₃ and H₂CO₃ in about the concentrations found in normal human plasma are illustrated in Table II.

9. Separation of Plasma from Centrifuged Blood.—The capillary tube I (Fig. 3) is connected to J, clamped in position as shown, and J and I are completely filled with mercury. The stopper is then removed from the centrifuge tube, and the latter is held so that the tip of I extends below the protecting layer of oil. With M at the lower level, K is opened and the plasma withdrawn into J. The pinch-cock is then placed in position on the outlet tube of J, I is removed, and M placed in its upper support. Samples for analysis are withdrawn as described above.

10. Preparation of Mercury and Apparatus Used for Collecting and Centrifugating Blood.—Commercial "redistilled" mercury before it was used was washed by falling from a capillary through a 1 meter column of 10 per cent nitric acid, and then through columns of distilled water. After contact with blood or serum, mercury was washed twice by shaking with distilled water, and was then passed through the nitric acid and water towers. Before use it was tested for the presence of alkali or acid by shaking a few cc. in a test-tube with water containing brom-cresol purple and phenol red. Glassware and rubber for use in contact with blood were cleaned, thoroughly rinsed with distilled water, and dried by drainage and evaporation at room temperature. When rapid drying of tonometers was necessary, they were rinsed with alcohol and ether of tested neutrality and dried in an air current.

Saturation.

We have used two processes to bring blood into equilibrium with gas mixtures of known composition. In both of them measured volumes of the gases were introduced with the blood into tonom-
eters, which were then revolved in a water bath at 38° until equilibrium was attained. In one process, however, the calculation of final gas tensions was based on direct gas analyses, the gas and

TABLE II.
Loss of CO₂ from BHCO₃, H₂CO₃ Solutions during Standing and Centrifugating.

Change in pH was used as an index of CO₂ loss. A 0.03 M NaHCO₃ solution containing phenol red was saturated at 38° with air containing CO₂ at 50 mm. tension. 20 cc. portions of the solution were then transferred under oil to centrifuge tubes and treated as outlined.

<table>
<thead>
<tr>
<th>No.</th>
<th>Initial pH</th>
<th>Final pH</th>
<th>Change pH</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>7.35</td>
<td>7.35</td>
<td>0.0</td>
</tr>
<tr>
<td>2</td>
<td>7.35</td>
<td>7.37</td>
<td>+0.02</td>
</tr>
<tr>
<td>3</td>
<td>7.36</td>
<td>7.44</td>
<td>+0.08</td>
</tr>
<tr>
<td>4</td>
<td>7.36</td>
<td>7.52</td>
<td>+0.10</td>
</tr>
<tr>
<td>5</td>
<td>7.35</td>
<td>7.37</td>
<td>+0.02</td>
</tr>
<tr>
<td>6</td>
<td>7.36</td>
<td>7.36</td>
<td>0.0</td>
</tr>
</tbody>
</table>

blood being separated at the end of saturation and analyzed separately. In the other process, which was usually employed when volumes of blood exceeding 30 cc. were used, direct analyses
of the gas phase were not made. The gas mixture was made up with especial accuracy, and the changes in its O₂ and CO₂ content during saturation of the blood were estimated from the amounts of these gases given off or taken up by the blood, which was analyzed both before and after its exposure to the gas mixture. With this procedure, separation of the gas phase in condition for analysis after saturation was unnecessary.

The oxygen (commercial) used was tested for purity by absorption with pyrogallol. The CO₂ from a Kipp apparatus, was tested by absorption with KOH solution. The hydrogen, sometimes commercial, sometimes from a Kipp apparatus, was analyzed for oxygen by absorption with pyrogallol in a Haldane apparatus. Nitrogen (commercial) was analyzed for contaminating oxygen in the same manner. When air was introduced it was freed of CO₂ by passage through a tower filled with moist sodium hydroxide "shells". This treatment reduced the CO₂ content of laboratory air to 0.01 per cent or less. In detail the two procedures for saturation of the blood were carried out as follows.

First Saturation Method. Final Tensions Determined by Analysis of Gas Phase.—The tonometer used (No. 1, Fig. 3) was a modification of a form introduced by Fridericia (1920), and consisted of a relatively small vessel (5 to 30 cc.); just sufficient to hold the blood, connected by a rubber tube of 6 mm. inner diameter to a larger vessel (of about 300 cc.). A length of about 3 cm. of rubber tubing was left between the glass parts. In order to fill the tonometer with the desired gas mixture, the tonometer was connected with the gas manifold at T (Fig. 3), and the air was drawn out through E. When the saturation was to be performed at an oxygen tension lower than that of air, nitrogen or hydrogen was then twice admitted, withdrawn as completely as possible, and readmitted. It was finally again drawn out, this time only until the pressure was reduced to about half an atmosphere. From the gas burettes through A and C sufficient CO₂ and O₂ were then admitted to give the desired tensions of these gases (see Equations 3 and 5 below). Finally, nitrogen or hydrogen was admitted until atmospheric pressure was attained.

The tonometer was then rotated horizontally in the bath, the blood being so distributed between the two chambers that the ratio of blood to gas volume was about the same in both. (A
The cock of the tonometer was opened near the surface of the water several times at intervals of a few minutes, until no more gas bubbles escaped. At each opening the tonometer was held so nearly upright that all the blood drained into the lower chamber; it was redistributed before the tonometer was clamped back into place.

The time required to attain equilibrium between a gas mixture and blood is dependent on the time necessary for the tonometer contents to reach bath temperature, on the relation of gas volume to liquid surface, on the invasion coefficient of the gas (Bohr, 1905, b), and, also, when the gases combine with substances in the blood, on the rate of combination. Under the conditions above outlined, about 15 minutes sufficed for the production of equilibrium when only CO₂ changes were involved, while 30 to 40 minutes were required when the oxygen tension of the blood was greatly reduced by the process.

Because the Δ of Equation 3 can be estimated only approximately, the gas tensions obtained at the end of a single saturation can be only approximately predetermined. Since the exact final tensions are accurately found by gas analyses, however, approximate predetermination of them is usually all that is needed (when, for example, data are being obtained for a curve).

When, however, it was desired to bring the blood gases to an exactly predetermined final tension, exposure in the tonometer was repeated once; and, when large tension changes were involved, twice. After the first exposure the tonometer was held upright in the bath until all the blood was in the lower chamber, which was then clamped off from the upper. The gas mixture in the latter was then drawn out and replaced as described above, with a mixture of the exact tension desired.

When the final saturation was complete, the tonometer was held upright in the bath until the blood had drained as completely as possible into the lower chamber. The latter was next separated from the upper chamber by screwing two clamps upon the rubber connecting tube. One clamp was attached near each glass part, that nearest the lower chamber being screwed down first, to avoid the compression of gases over the blood that would result if the upper clamp were fastened first. The tonometer was then raised
so that only the lower chamber remained in the bath, and the rubber tube was cut with scissors just below the upper clamp, leaving about 2 cm. of the tube projecting above the lower.

Into the upper, gas-containing chamber, about 50 cc. of mercury were run under pressure through the 3-way cock. The gas was thereby put under positive pressure, which facilitated drawing samples into the Haldane apparatus for analysis, and prevented the possibility of entrance of atmospheric air by leakage.

While the separated lower chamber still remained in the bath its 3-way cock was connected with a mercury leveling bulb. The clamp on the rubber outlet tube was then opened for a few seconds, while mercury was run into the chamber from below until the blood had risen into the rubber tube and displaced all the gas in the chamber. The clamp on the tube was then closed; the chamber was taken from the bath, clamped on a ring-stand, and removed for analysis of the blood.

Blood samples for analysis were drawn exactly as from the sampling tube described on page 131 (J, Fig. 3). In order to make the pipette tip fit tightly into the rubber outlet tube of the chamber, it was usually necessary to encircle the tip with a rubber ring cut from a section of tubing. This ring was lubricated with vaseline to facilitate fitting it into the rubber tube of the blood container.

Second Saturation Method. Final Tensions Estimated from Analyses of the Blood.—In the second method, used for larger volumes of blood than the first, a tonometer, modelled like a Barcroft gas sampling tube, with a single chamber (No. 2 of Fig. 3) of about 800 cc. capacity was employed. The volume of each tonometer, determined by weighing the water it held, was etched upon it. The gas mixture was made in the tonometer, as described in the first method, by admitting measured volumes of oxygen and CO₂, calculated by Equations 3 and 5 below. The measured volume of analyzed blood, usually about 75 cc., was drawn in through the lower cock after the significant gases had been introduced, and before the pressure was finally adjusted by admission of air, hydrogen, or nitrogen. In this case, the final total pressure at room temperature was kept 80 mm. below atmospheric by admitting the last gas through the mercury pressure regulator shown in Fig. 3. Otherwise, since the cocks were not opened
during saturation, they were likely to be forced out by the pressure which developed as the gases within warmed up in the bath. The tonometers were in all cases made of Pyrex glass.

When it was necessary to know exactly the final tension at equilibrium, but not necessary that this tension be precisely at a predetermined point, one saturation was sufficient. The initial gas mixture was prepared according to Equations 3 and 5 below, to give approximately a desired final tension, and the exact final tension was calculated after analysis of the saturated blood by Equation 4.

When, however, the plan of the experiment made it desirable to fix the final tension of CO₂ or O₂, or both, at exact, predetermined points, either two or three successive saturations were performed on the same blood in different tonometers. The tension for the first saturation was calculated according to Equation 3, while for the second and third, the gases were measured into the tonometers in such proportions as to produce the exact tension desired. The total number of saturations was two, if the tension change in the blood was slight, such as the change from 45 to 40 mm. of CO₂ tension; while three equilibrations were used if the change was larger, as when blood at 40 mm. was changed to 20 or 60. Analyses of the blood were performed after each of the last two saturations. Usually both results were identical. If there was a difference, the slight effect on the final tension was calculated by Equation 4.

Transfer of Blood from Tonometer to Tonometer in Second Saturation Method.—The receiving tonometer was evacuated, and the desired amounts of CO₂ and O₂ were run in, together with enough inert gas (H₂ or N₂) to make about half an atmosphere of pressure. The two tonometers were connected below by a capillary U-tube, the delivering tonometer having been wrapped in a towel wet with water at 38° as soon as it was removed from the bath. The upper cock of the delivering tonometer was opened and the connecting tube was filled with blood by manipulation of the lower cocks. Then all but a few drops of the blood was drawn over into the receiving tonometer. Sufficient inert gas was finally admitted to raise the pressure to B—80 mm.

Transfer of Blood from Tonometer to Final Container in Second Saturation Method.—One 3-way cock of the tonometer was lifted...
from the bath and connected with the mercury-filled receiving tube (J, Fig. 3), and the connections were filled with mercury. The tonometer was then inverted and placed upright in the bath, with the mercury-filled receiving vessel below and the upper end projecting from the bath. The upper cock was opened to the air, and the blood was drawn down into the receiving tube. In some instances the exchange was quickly performed outside the bath, the tonometer having been wrapped with a towel before removal from the water.

Calculations.

The formulas used in the calculations were developed as follows:

Let \( p_f \) = final tension in mm. of mercury, of a specified gas (CO\(_2\) or O\(_2\)) in tonometer at end of saturation.

\( p_i \) = initial tension of the specified gas in tonometer at the beginning of saturation.

\( \Delta \) = increase in total (free and combined) millimolecular concentration of the specified gas in the blood caused by changing the blood from its original state to that at the end of saturation. (\( \Delta \) is negative if the concentration of the gas in the blood decreases during the saturation.)

\( T_{rn} \) = absolute temperature of tonometer during saturation.

\( T_{burett} \) = absolute temperature of burette from which gas is measured into tonometer.

\( V_{rn} \) = cc. total volume content of tonometer.

\( V_{burett} \) = cc. volume of the specified gas measured over water in the burette, at barometric pressure and \( T_{burett} \), which must be transferred to the tonometer to give therein \( p_i \) tension of the gas at \( T_{rn} \).

\( V_{bl} \) = cc. volume of blood in tonometer during saturation.

\( V_{rn} - V_{bl} \) = cc. volume of gas space in tonometer during saturation.

\( B \) = barometric pressure in mm. of mercury.

\( W \) = vapor tension of water in mm. of mercury.

If we place the volume of CO\(_2\) or O\(_2\) absorbed by the blood, equal to the volume lost by the gas phase to the blood during saturation, we obtain an equation from which may be calculated the initial tension necessary to secure a given final tension or the final tension resulting from a given initial one.

The number of cc. of CO\(_2\) or O\(_2\), reduced to \( 0^\circ, 760 \) mm., absorbed by the blood during saturation is equal to the product of the total cc. of blood times the volume of gas absorbed by each
cc. This product is $V_{bl} \times 0.0224 \Delta$. (The factor 0.0224 is the number of cc. of gas, reduced to 0°, 760 mm., contained in 1 cc. of a millimolecular solution of the gas. Consequently 0.0224Δ is the volume of gas, reduced to 0°, 760 mm., absorbed by each cc. of blood. The equations may be transformed into terms of volumes per cent of gas by substituting 0.01 for 0.0224.)

The total initial volume of the specified gas, reduced to 0°, 760 mm., in the gas space of the tonometer is $(V_{in} - V_{bl}) \frac{P_i}{760} \frac{273}{T}$, and the final volume of the gas, at 0°, 760 mm., is $(V_{in} - V_{bl}) \frac{P_f}{760} \frac{273}{T}$. The volume of gas, at 0°, 760 mm., lost from the gas phase to the blood during saturation is the difference between the two, or $(V_{in} - V_{bl}) \frac{P_i - P_f}{760} \frac{273}{T}$. Placing the above two expressions equal to each other we have

$$0.0224 \Delta V_{bl} = (V_{in} - V_{bl}) \frac{P_i - P_f}{760} \frac{273}{T}$$

Volume of O₂ or CO₂ gained by blood. Volume of O₂ or CO₂ lost by gas phase.

In order to find the CO₂ tension, $P_i$ in the tonometer with which we must begin saturation to reach the desired final tension $P_f$ we solve Equation 1 for $P_i$ and obtain Equation 2.

$$P_i = P_f + \left(760 \times 0.0224\Delta \frac{T_{in}}{273} \frac{V_{bl}}{V_{in} - V_{bl}}\right)$$

When the numerical constants are combined this becomes

$$P_i = P_f + 0.0624 \Delta \frac{T_{in}}{273} \frac{V_{bl}}{V_{in} - V_{bl}} = P_f + 19.4 \Delta \frac{V_{bl}}{V_{in} - V_{bl}} \quad \text{when } T_{in} = 311° = 38°C.$$
Blood Saturation with Gas Mixtures

\[ p_f = p_i - 0.0624 \frac{\Delta T_{in}}{V_{in} - V_{bl}} \frac{V_{bl}}{V_{in} - V_{bl}} \]

\[ = p_i - 19.4 \Delta \frac{V_{bl}}{V_{in} - V_{bl}} \text{ when } T_{in} = 311^\circ = 38^\circ C. \] (4)

For calculation of the volume \( V_{\text{burette}} \) of gas which must be measured in the burette (Fig. 3) at atmospheric pressure and transferred to the tonometer to give therein the initial tension \( p_i \), we have used Equation 5, which is developed by placing equal to each other the expressions indicating the volume of gas measured in the burette and in the tonometer, respectively, both being reduced to 0°, 760 mm. We then have

\[ V_{\text{burette}} = \frac{273}{760} \times \frac{B-W_{\text{burette}}}{T_{\text{burette}}} \times (V_{in} - V_{bl}) \frac{p_i}{760} \frac{273}{T_{in}}, \text{ whence} \]

\[ V_{\text{burette}} = (V_{in} - V_{bl}) \frac{p_i}{B-W_{\text{burette}}} \frac{T_{\text{burette}}}{T_{in} T_{tn}}, \text{ or} \]

\[ = \frac{p_i (V_{in} - V_{bl})}{T_{in}} \frac{T_{\text{burette}}}{B-W_{\text{burette}}} \] (5)

Solving Equation 5 for \( p_i \) we obtain in Equation 6 the tension at bath temperature given by the gas volume, \( V_{\text{burette}} \), measured into the tonometer.

\[ p_i = V_{\text{burette}} \frac{T_{in}}{V_{in} - V_{bl}} \frac{B-W_{\text{burette}}}{T_{\text{burette}}} \] (6)

We have found it convenient to use Equation 5 in the second of the two forms given above, for the reason that it places in one group the factors \( p_i, T_{in}, \) and \( (V_{in} - V_{bl}) \) which are independent of temporary room conditions and can therefore be calculated in advance, while the factors \( T_{\text{burette}} \) and \( (B - W_{\text{burette}}) \), which must be determined at the moment of measurement, are placed together in a second group.

Approximate Estimation of \( \Delta \) for Calculation of Initial Tensions of First Saturation.—This estimate was made by means of the average absorption curve of the kind of blood used. The manner in which the estimate was made is shown by an example. In the venous blood drawn from the horse used in most of our experiments the \( CO_2 \) tension was constantly in the neighborhood of 45 mm. The
average absorption curve of the animal's blood showed that changing the CO₂ tension produced approximately the following changes in CO₂ content:

<table>
<thead>
<tr>
<th>( p_f ) (mm.)</th>
<th>CO₂ content (mm.)</th>
<th>( \Delta ) (mm.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>15.0</td>
<td>-6.6</td>
</tr>
<tr>
<td>40</td>
<td>20.5</td>
<td>-1.1</td>
</tr>
<tr>
<td>45</td>
<td>21.6</td>
<td>0.0</td>
</tr>
<tr>
<td>60</td>
<td>23.5</td>
<td>+1.9</td>
</tr>
<tr>
<td>80</td>
<td>25.2</td>
<td>+3.6</td>
</tr>
</tbody>
</table>

While the CO₂ content of the blood as drawn varied at times considerably from 21.6 mm., the values of \( \Delta \) caused by given changes in tension remained fairly constant.

**Exact Determination of \( \Delta \) for Calculation of Final Gas Tensions.** This determination was made by comparison of the analyses of the blood after each of the last two saturations.

**Example:**

<table>
<thead>
<tr>
<th>Equilibration No.</th>
<th>CO₂ content of blood (mm.)</th>
<th>( \Delta ) (mm.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>21.6</td>
<td>-0.4</td>
</tr>
<tr>
<td>3</td>
<td>21.2</td>
<td></td>
</tr>
</tbody>
</table>

**Limits of Error in Determination of Final CO₂ and O₂ Tensions.**

1. **By Analysis of Gas Phase at End of Saturation (First Method).** The analytical error of CO₂ and O₂ determinations by the Haldane apparatus is ordinarily about ± 0.02 volumes per cent. The tension corresponding to this at 760 mm. and 38° is \( \frac{0.02}{100} = 0.14 \text{ mm. of mercury} \). It appears probable that the error of estimating gas tensions produced by the "First saturation method" may be kept below 0.2 mm., since errors are practically excluded in separating the gas phase for analysis. As a matter of fact, results shortly to be published indicate that this degree of accuracy can be obtained quite consistently.

2. **By Analysis of Blood Before and After Saturation with Known Gas Mixtures (Second Method).** The final tension is determined by Equation 4, which for 38° is
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\[ p_f = p_i + 19.4 \Delta \frac{V_{bl}}{V_{tn} - V_{bl}} \]

In determining the possible error in calculating \( p_f \), we estimate and add the maximum errors in the experimental determinations of \( p_i \) and of the value \( 19.4 \Delta \frac{V_{bl}}{V_{tn} - V_{bl}} \), respectively, the latter value representing the correction which must be applied to \( p_i \) as the result of the loss or gain of significant gas by the blood during saturation.

\( p_i \) is determined by the amount of gas measured into the tonometer. From Equation 5 we have

\[ p_i = \frac{V_{burette}}{V_{tn} - V_{bl}} \times (B - W_{burette}) \times \frac{T_{burette}}{T_{tn}}. \]

At the usual conditions, \( T_{burette} = 20^\circ + 273, T_{tn} = 38^\circ + 273, R = 760, R - W = 743, V_{tn} = 800, V_{bl} = 75 \), we have \( p_i = V_{burette} \times 1.17 \). 1 cc. of gas, measured in the burette (Fig. 3), therefore corresponds to a tension of about 1.2 mm. of mercury in the tonometer. The gas can be measured with an error not exceeding 0.10 cc., so that \( p_i \) can be fixed within about 0.10 \( \times \) 1.2 = 0.12 mm. by measurement. The errors in the other factors, \( \text{viz.} (V_{tn} - V_{bl}), (B - W), \) and \( \frac{T_{burette}}{T_{tn}} \) are relatively so small as to be negligible.

The error in determining the value of the term \( 19.4 \Delta \frac{V_{bl}}{V_{tn} - V_{bl}} \) may be estimated as follows: In our experiments the blood occupied about one-tenth as much space as the gases in the tonometer, \( \frac{V_{bl}}{V_{tn} - V_{bl}} = 0.1 \). Substituting 0.1 for the factor \( \frac{V_{bl}}{V_{tn} - V_{bl}} \) in Equation 4, for \( 38^\circ \), we have \( p_f = p_i - 1.94 \Delta \). That is, a change of 1 mm. in the value of \( \Delta \) for \( \text{CO}_2 \) or \( \text{O}_2 \) causes a change of about 1.9 mm. in the tension of the gas at \( 38^\circ \). The analytical error of our blood gas determinations is about \( \pm 0.05 \) mm.; and \( \Delta \) represents the difference between two determinations, so that its possible error would be twice as great, or \( \pm 0.1 \) mm.
The latter would cause an error of $1.9 \times 0.1$, or about 0.19 mm.
in the calculation of the value, $1.94 \Delta \frac{V_{bl}}{V_{tn} - V_{bl}}$.

The total error in the determination of the final tension in the
manner indicated by the equation $p_f = p_i + 19.4 \Delta \frac{V_{bl}}{V_{tn} - V_{bl}}$ we
therefore estimate as approximately $0.12 + 0.19 = 0.3$ mm. of
O$_2$ or CO$_2$ tension. This is of the same order of magnitude as the
error involved in estimating the final tension by analysis of the
tonometer gas, discussed above.

The error in the calculation from $\Delta$ may be reduced in either of
two ways. (1) Since the error is chiefly due to the factors involved
in the term $\Delta \frac{V_{bl}}{V_{tn} - V_{bl}}$, it may be reduced by reducing $V_{bl}$, the
volume of blood used, and therefore the factor $\frac{V_{bl}}{V_{tn} - V_{bl}}$.

(2) When the final tension has been estimated by Equation 4, the
blood may be saturated once more, at exactly this tension. The
error is thereby practically reduced to that involved in fixing
$p_i$, which is only 0.1 mm. of tension. This procedure has been
followed when maximum accuracy has been required in the
saturation of large volumes (50 - 100 cc.) of blood.

The following experiment indicates that with the apparatus
used the two methods for fixing initial tensions agree within their
limits of error:

Into two tonometers, in which the pressure had been reduced to about
60 mm., measured amounts of CO$_2$ were introduced from burette A (Fig. 3).
Air was admitted until the pressure in the tonometers was brought to
atmospheric. Samples of the gas mixture were then displaced from the
tonometers into a Haldane air analysis apparatus and analyzed for CO$_2$.
CO$_2$ tensions calculated from the volume of CO$_2$ introduced (Equation 5)
and the tensions calculated from the results of the gas analyses, respect-
ively, are shown in Table III.
### TABLE IV.

<table>
<thead>
<tr>
<th>Formula No.</th>
<th>Use of formula.</th>
<th>Formula in terms of [H⁺] and K'.</th>
<th>Formula in terms of pH and pK'.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Calculation of [H₂CO₃] from p.</td>
<td>mm. [H₂CO₃] = \frac{\alpha p}{760 \times 0.0224} = 0.0587 \alpha p</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Calculation of [BHCO₃] from [CO₂] and p.</td>
<td>mm. [BHCO₃] = [CO₂] - 0.0587 \alpha p</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Calculation of [H⁺] or pH from [CO₂] and p.</td>
<td>[H⁺] = K' \frac{0.0587 \alpha p}{[CO₂] - 0.0587 \alpha p}</td>
<td>pH = pK' + \log \frac{[CO₂] - 0.0587 \alpha p}{0.0587 \alpha p}</td>
</tr>
<tr>
<td>4</td>
<td>Calculation of K' or pK' from [H⁺] or pH and [CO₂].</td>
<td>K' = \frac{[H⁺][CO₂] - 0.0587 \alpha p}{0.0587 \alpha p}</td>
<td>pK' = pH - \log \frac{[CO₂] - 0.0587 \alpha p}{0.0587 \alpha p}</td>
</tr>
<tr>
<td>5</td>
<td>Calculation of CO₂ tension from pH and [CO₂].</td>
<td>p = \frac{[CO₂]}{0.0587 \alpha \left(\frac{K'}{[H⁺]} + 1\right)}</td>
<td>p = \frac{[CO₂]}{0.0587 \alpha \left(10^{pH - pK'} + 1\right)}</td>
</tr>
<tr>
<td>6</td>
<td>Calculation of [BHCO₃] from pH and [CO₂].</td>
<td>[BHCO₃] = [CO₂] \frac{1}{1 + \frac{[H⁺]}{K'}}</td>
<td>[BHCO₃] = [CO₂] \frac{1}{1 + 10^{pK' - pH}}</td>
</tr>
</tbody>
</table>

*In order to use formulas with [CO₂], [BHCO₃], and [H₂CO₃] expressed in terms of volumes per cent of CO₂ instead of mm. concentration, replace the factor 0.0587 by the factor \frac{100}{760} or 0.1316.*
That the final tension can be set with a similar degree of accuracy is indicated by the constancy of the final CO₂ contents obtained in the accompanying second paper of the series. In the horse blood used a deviation of 0.7 volume per cent or 0.3 mm. in CO₂ content corresponds to a deviation of 1 mm. in CO₂ tension.

Formulas for Calculating pH, [H⁺], pCO₂, [BHCO₃], and [H₂CO₃] from Data Usually Obtained by Direct Determination.

We have used certain rearrangements of Henderson's and Hasselbalch's equations so frequently that it appears desirable, in order to avoid subsequent repetition, to record them here in connection with the description of technique.

The constants are given in terms of millimolecular (mM.) concentration, rather than in volumes per cent of gas, for the reason that comparison of concentration changes not only in oxygen and carbon dioxide, but also in electrolytes, such as chlorides and alkali protein compounds, has been necessary in the studies outlined, and a single unit of concentration that can be used throughout is desirable.
The following symbols are used:

\[ [\text{CO}_2], [\text{BHCO}_3], [\text{H}_2\text{CO}_3] = \text{millimolecular concentration of total CO}_2, \]
\[ \text{BHCO}_3, \text{and H}_2\text{CO}_3, \text{respectively}. \]
\[ \alpha = \text{solubility coefficient of CO}_2 \text{ at } 38^\circ. \]
\[ p = \text{tension of CO}_2 \text{ in millimeters of mercury.} \]
\[ K' = \text{constant by which the ratio } \frac{[\text{H}_2\text{CO}_3]}{[\text{BHCO}_3]} \text{ must be multiplied to give} \]
\[ [\text{H}^+]. \text{Theoretically } K' = \frac{K}{\gamma} \text{ where } K \text{ is the dissociation} \]
\[ \text{constant of H}_2\text{CO}_3, \gamma \text{ the fraction of BHCO}_3 \text{ dissociated into} \]
\[ \text{B}^+ \text{ and HCO}_3^- \text{ (Hasselbalch, 1917).} \]
\[ pK' = -\log K' \text{ (Hasselbalch, 1917).} \]

The values in Table V have been used. For the constants \( \alpha, K', \) and \( pK' \), the values in Table V have been used.

In Formula 2 the \( [\text{BHCO}_3] \) is calculated by subtracting from the total \( [\text{CO}_2] \) the \( [\text{H}_2\text{CO}_3] \), which is calculated from the \( \text{CO}_2 \) tension according to Formula 1. Formula 3 is Henderson's familiar \( [\text{H}^+] = K' \frac{[\text{H}_2\text{CO}_3]}{[\text{BHCO}_3]} \) with \( [\text{H}_2\text{CO}_3] \) and \( [\text{BHCO}_3] \) calculated according to Formulas 1 and 2.

Formula 4 is obtained by obvious rearrangement of Formula 3. Formula 5 is obtained by solving Formula 3 or 4 for \( p \). Formula 6 is obtained by substituting the value of \( p \) from Formula 5 for \( p \) in Formula 2.

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